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THE FORMATION OF THE FIRST POLAR SPINDLE IN THE EGG OF BUFO LENTIGINOSUS.

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In a previous paper on "The Maturation and Fertilization of the Egg of *Bufo lentiginosus*" (King, 10), the formation of the first polar spindle and the subsequent divisions of the chromosomes were very incompletely described owing to a lack of material showing the details of these processes. During the spring of 1899, a large number of toads were collected soon after they had emerged from their hibernation, and from three of them sufficient material was obtained to give a more complete history of the late maturation processes. A short account of my study of this period in the development of the egg has already appeared (11); a detailed account is given in the present paper.

I. Material and Methods.

As soon as possible after the toads were captured they were killed by pithing and the body opened at once to ascertain the condition of the ovaries. In a great majority of cases the eggs were found free in the cœlomic cavity and were, therefore, of no use for the purpose intended, as previous investigations had shown that eggs which have broken through the wall of the ovary invariably contain a fully formed maturation spindle lying at the periphery near the center of the black hemisphere.

In several instances, all of the eggs were still attached to the walls of the ovaries when the toad was killed. In these cases some of the eggs were put at once into a dish of fresh spring water and the rest were left in the body of the female which was

kept in a moist chamber. A few eggs from each of these series were then fixed at intervals of ten minutes for a period of several hours. By opening an egg under a dissecting lens after it has been taken from the fixing solution and put into 50 per cent. alcohol, one can tell definitely whether the late maturation processes have begun or not; for, if the nuclear membrane in still intact, the nucleus retains its rounded form and can be readily separated from the rest of the egg contents. If one hour after the toad is killed, an examination of freshly fixed eggs shows that the nucleus is still intact, the entire set of eggs can be discarded, as it has been found that further development does not take place unless the germinal vesicle breaks down previous to this time, although the eggs, whether kept in water or in the body of the female, show no signs of disintegration for many hours.

In one case the germinal vesicle was just breaking down when the eggs were first examined under the dissecting lens; in another set of eggs the germinal vesicle could no longer be dissected out half an hour after the toad was killed. These two lots of eggs gave overlapping series of stages which corresponded in every respect. A third set of eggs showed no signs of the germinal vesicle when first examined, and when sectioned showed maturation processes identical with those taking place in eggs which had been developing in water for several hours.

In all these three sets of eggs, the first polar body was given off in the normal position and apparently in the normal manner before the eggs showed any signs of disintegration. No difference was noticed in the development of eggs which had been put into water and those which had been left in the body of the toad. It does not seem, therefore, that such unusual conditions interfere at all with the late maturation processes provided these processes have started before the normal conditions are changed. No attempt was made to fertilize these eggs artificially, as it has never been found possible to fertilize either the eggs of *Bufo* or of *Rana* until they have received the thick jelly-like membrane which is secreted around them in the oviducts.

In all cases the eggs were fixed in corrosive-acetic and stained with a combination stain of borax carmine and Lyon's blue as described in a previous paper (King, 10).

II. The Disintegration of the Germinal Vesicle.

I have already given in detail a description of the early stages in the breaking down of the germinal vesicle, and as this new material confirms but adds nothing to that description, it will be necessary to give only a brief account of the changes in the egg directly preceding the formation of the spindle.

At the end of the hibernation period the germinal vesicle lies in the upper hemisphere of the egg. It is round in outline and contains a large number of nucleoli which usually form a ring enclosing the chromatin threads. A layer of granular substance staining differently from the cytoplasm, surrounds the lower pole of the germinal vesicle and extends half way up each side. This substance appears homogeneous at first and then becomes a compact, fibrous band of uniform thickness. I have called this band a "line of radiation," because, as soon as the nuclear membrane has disappeared in this region, the karyoplasm of the nucleus forms into coarse granules and a pronounced radiation extends up into the nuclear substance from the entire length of the fibrous band below. The karyoplasmic granules soon become smaller and more numerous and finally disappear entirely, while the radiation from below continues to increase and often extends nearly to the upper surface of the egg. The rays forming this radiation are very fine, and their outer ends run, apparently, into the coarse network which comes to fill the entire space formally occupied by the germinal vesicle. During these changes, the nucleoli have lost their power of staining and have begun to disintegrate.

When the nuclear membrane breaks down, twenty-four chromosomes, arranged in pairs, are scattered throughout the upper part of the nuclear space. The ends of each pair then unite to form a closed ring near which a small aster usually appears. The aster has no centrosome and its rays rarely touch the chromatin ring. At the next stage, when the radiation from below has reached its greatest extent, the asters and the chromatin rings entirely disappear. Later, when the radiation has begun to decrease, a large number of small round chromatin granules are found near or on the line of radiation which has been gradually shortening during this period. When the chromatin granules

first appear they stain very faintly, but they soon take the deep carmine stain characteristic of chromatin, and then fuse into several large, irregular clumps.

III. The Formation of the First Polar Spindle.

The line of radiation, shortly after the appearance of the chromatin granules, is shown in Fig. 1. It is a short, fibrous band with its ends, usually, though not invariably, slightly curved in towards the center of the egg. This structure, which is to become the first polar spindle, lies some distance below the surface of the egg in a small accumulation of granular substance formed, possibly, from the karyoplasm of the germinal vesicle. Its longitudinal axis may be either parallel or oblique to the surface of the egg, the latter position being the more common. Running out in every direction from the compact meshwork of fibers are numerous fine, thread-like rays which are longest and most numerous at the middle of the forming spindle where they extend out between the yolk spherules and seem to be continuous with the cytoplasmic network of the egg.

Collected near the middle of the spindle is a mass of small chromatin granules which are of uniform size and stain but faintly in comparison with the chromosomes of an earlier and of a later period. There is a very large number of these granules and it is quite impossible to count them satisfactorily; two other sections of the same egg each show as many granules as are shown in Fig. 1.

The nucleoli from the germinal vesicle appear at this period as irregular, yellowish green, refractive bodies which are scattered throughout the upper hemisphere of the egg, often lying quite close to the spindle. They disappear at different times in different eggs. Sometimes they have all been absorbed before the chromosomes have divided; sometimes they can still be found after the first polar body has been given off. I have never found any traces of them, however, after the spermatozoön has entered the egg.

Not more than fifteen minutes after the stage of Fig. 1, the chromatin granules begin to fuse into irregular-shaped clumps. The number and size of these clumps vary greatly in different eggs, in some cases there are but four or five of them, in others

at least twenty. Owing, probably, to their greater volume, these larger masses always stain much more intensely than do the small granules. Meanwhile the spindle has lost its uniform diameter and has become much thicker in the middle where the meshwork of fibers appears more distinct and more regular. The spindle soon becomes barrel-shaped and its fibers are quite clearly defined in the middle region but not at the poles (Fig. 2). The radiation from the spindle disappears entirely except at the poles where it forms distinct asters; some of the rays are very long and cross each other at the equator of the spindle. During its migration towards the upper pole of the egg the spindle shortens somewhat and gradually becomes more slender and pointed, a phenomenon seen by Van Name (17) in the eggs of Planarians, by Korschelt (12) in *Ophryotrocha*, by Griffin (8) in *Thalassema*, and by Boveri (1) in *Ascaris*.

At no stage in the formation of the spindle or in its later history can any centrosome be found in the polar asters. As the spindle becomes more pointed, the rays converge more sharply at the poles, but even when the radial systems are best developed (Figs. 2, 3), the rays appear to run into each other in the center of each aster and there is not the slightest trace of any kind of a central body. Carnoy and Lebrun (2) in their study of the batrachian egg, Eismond (5) in his work on *Siredon* and *Triton*, Fick (6) in studying the maturation of the Axolotl egg, and *Sobotta* (15, 16) in working on the egg of the mouse and of *Amphioxus*, have all failed to find a centrosome in the asters of the polar spindles. If such a structure is normal in these eggs and also in the egg of *Bufo lentiginosus*, methods of fixation and staining which have so clearly demonstrated its presence in other eggs are totally inadequate in these cases to show the slightest trace of it.

At the stage of Fig. 3, the small chromatin granules have entirely disappeared. Whether they have all gone into the large chromatin clumps or whether some have been absorbed by the cytoplasm cannot be determined. At this time the number of large chromatin masses still varies slightly in different eggs; in some cases there are nine such clumps of chromatin, in others at least fifteen. These chromatin masses are, for the most part, scattered irregularly along the spindle fibers, occasionally, however, one or more of them can be seen entirely outside of the

spindle (Fig. 4, *CM*). Isolated masses of chromatin are sometimes found near the spindle at a much later period when the chromosomes are at the equator preparing to divide. They have entirely disappeared by the time the first polar body is given off, possibly serving as food for the cytoplasm as suggested by Gardiner (7).

During the next half-hour, the irregular chromatin masses change into chromosomes with a definite shape. The change does not take place at the same time in all of the chromatin clumps; in fact, until the chromosomes are arranged at the equator of the spindle ready to divide, they may be found in several different stages of development on the same spindle. Twelve chromosomes, one-half the number characteristic of the somatic cells of this species differentiate from the chromatin masses. The chromosomes are scattered over the entire spindle and are at first somewhat triangular in shape (Fig. 3), later they become rod-shaped structures which may lie with their long axis parallel, oblique, or even at right angles to the longitudinal axis of the spindle (Fig. 4). Sooner or later, however, the long axis of each chromosome comes to lie parallel with the spindle fibers and the chromosomes then have a rounded knob in the middle region and frequently also a smaller knob at each end (Figs. 4, 5). Later the middle knob becomes more prominent and the end knobs disappear (Fig. 5).

At the stage of Figs. 2–3 the asters at the spindle poles reach their greatest development. There are many long rays from each aster which run nearly parallel with the spindle fibers and cross each other at the equator of the spindle, and fewer and much shorter rays going out in other directions. Soon after this time the asters begin to degenerate. The shorter rays disappear first and by the time the spindle has reached the periphery of the egg there is not a trace of the radiation left. The spindle fibers then converge at the poles which are surrounded by a small accumulation of granular substance probably formed from the disintegrated rays (Fig. 7).

There is often a marked difference in the size of the chromosomes on the same spindle even when they are of exactly the same shape. One or two of the chromosomes may extend over one-third the length of the spindle, the others being not more

than one-half as large (Fig. 5). This difference is not found at a later period; for, when the chromosomes are arranged at the equator of the spindle ready to divide, they are considerably smaller than the chromosomes of an earlier period and are all, apparently, of the same size.

While the chromosomes are being arranged at the equator of the spindle they undergo further changes in form. The polar arms shorten considerably, while the thick knob at the middle increases in size and gradually spreads out laterally, thus forming two wing-like projections on the chromosomes (Figs. 6, 7). In proportion as the lateral wings grow larger the polar arms of the chromosomes become shorter and thinner, so that there can be no question but that this lateral growth takes place at the expense of the rest of the chromosome. In a dorsal view, the wings appear to be spread out flat on the spindle and the chromosome has the appearance of a cross in which the polar arms are somewhat longer than the equatorial arms (Fig. 6). In a lateral view, however, the wings are seen to be raised up from the spindle while the polar arms are extended along the spindle fibers. Carnoy and Lebrun (2) have applied the term "oiselet" to this stage in the development of the chromosome. The typical oiselet stage is followed by one in which the body of the "bird" gradually disappears while the wings constantly increase in size (Fig. 7). Very soon, all that is left of the original polar arms is a slight projection on each side of the angle formed by the meeting of the two wings (Fig. 8). In the succeeding stage every trace of the polar arms has disappeared and there are twelve broad V-shaped chromosomes arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle (Fig. 9). Usually, before this last stage is reached, the spindle has come to lie close to the surface of the egg and nearly radial in position. This is by no means invariably the case, however, as sometimes the spindle is still some distance below the surface of the egg when the chromosomes have divided in preparation for the first maturation division.

Fig. 6 shows part of a section of an egg fixed as soon as possible after the toad was killed. The spindle lies at the periphery of the egg and the chromosomes, with well-developed lateral wings, are at the equator. That this egg and others from the

same series are normal cannot be questioned. They show phenomena exactly similar to those seen in eggs that have been developing in water for some three hours, and leave no doubt but that the earlier processes described above are normal in spite of the unusual conditions to which many of the eggs were subjected.

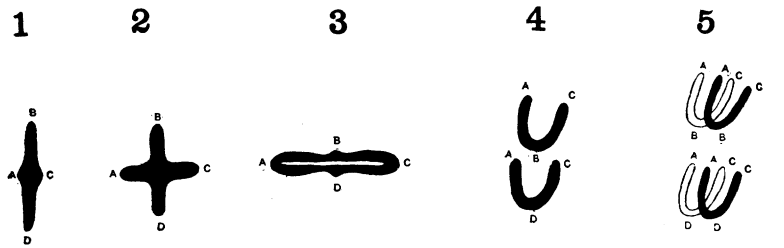
Four V-shaped chromosomes in which all traces of the polar arms have disappeared are shown in Fig. 9. The arms of the V's are broad flat plates which form a sharp acute angle with each other. There is, in this case, no sign of a splitting in any of the chromosomes which are all of the same size and shape and arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle, a characteristic arrangement of the chromosomes at this period. An equatorial section of a spindle in the same stage as Fig. 9, is seen in Fig. 10, where all twelve chromosomes are present. In this egg there are also found near the spindle a number of nucleoli which are in the process of disintegration.

Usually the first indication of any division of the chromosomes is seen at the stage of Fig. 11 when the polar arms have entirely disappeared and the chromosomes are broad V-shaped structures. At this time the ends of the V's often show a deep indentation (Fig. 11) indicating the longitudinal splitting of the chromosomes. Occasionally I have found the first division coming in at an earlier period before the entire disappearance of the polar arms. Such a division is seen in the chromosome at the left in Fig. 7. In all such cases the splitting is confined entirely to the lateral wings and never extends into the polar arms.

In the egg from which Fig. 12 was drawn, there are twenty-four V-shaped chromosomes which are similar to the twelve chromosomes in Fig. 10 in every respect except that they are much narrower. They have been produced, I believe, by a longitudinal division of the broad V-shaped chromosomes found at an earlier period. In some of the chromosomes shown in Fig. 12, the division for the second polar mitosis is seen. This second division of the chromosomes is not visible, at this stage, except in equatorial sections of the spindle. In longitudinal sections of the spindle the chromosomes always appear to be arranged in tetrad groups, one of which may be seen in Fig. 12. Such a group is, in reality, a pair of V-shaped chromosomes with

the angle of each V turned in towards the center of the spindle, the four ends of a pair of chromosomes projecting from the spindle give the appearance of a typical tetrad. The maturation divisions of the chromosomes are represented diagrammatically by text-figures 1–5.

In my previous paper, three sections from one egg (Figs. 25, 26, 27) were given in which the fully formed spindle lay some



Diagrams of the maturation divisions of the chromosomes in the egg of Bufo lentiginosus.

distance below the surface of the egg and the chromosomes were in the form of closed rings which were split longitudinally. This egg was undoubtedly abnormal and led to the wrong inference that these chromatin rings were identical with those found in the germinal vesicle just previous to its disintegration. If the split V-shaped chromosomes of Fig. 11 were to be spread out in the form of a ring and the second maturation division to take place before the halves of the ring separated, then exactly the same effect would be produced as previously illustrated in Figs. 25–27. I can only interpret the ring-shaped chromosomes in this abnormal egg — the one abnormality I have found in many hundreds of eggs sectioned — as due to a delay in the separation of the parts after the two divisions of the chromosomes had taken place.

According to Carnoy and Lebrun (2, 3) who have published a series of memoirs dealing with the development of the germinal vesicle and the formation of the polar bodies in the eggs of various Batrachia, the chromatin filaments in the egg of Salamander, *Alytes*, *Triton*, *Bufo* and *Rana* arise from repeated resolutions of the nucleoli in the germinal vesicle. As my own work on *Bufo* began with the fully formed egg taken from the animal just before the beginning of the hibernation period, I have not yet seen this

resolution of the nucleoli into chromatin threads. In all the eggs that I have examined in which the germinal vesicle was intact, the chromatin was always in the form of distinct chromosomes. These chromosomes had no connection whatever with the large round nucleoli which, with the combination stain used, always stain a deep blue while the chromatin invariably takes the carmine. I have frequently noticed, however, that many of the chromosomes end in small granules which take the same stain as the chromatin and that there are a number of similar granules scattered throughout the nucleus. Recent work on various forms has shown that unquestionably the term nucleolus has been applied to many different kinds of structures in the germinal vesicle. As a general term used to cover any definite structures in the germinal vesicle other than chromosomes, linin and karyoplasm, it may, perhaps, be fitly applied both to the large rounded structures (which I consider the only true nucleoli in the germinal vesicle) and to the smaller granules which stain like chromatin and which I believe to be chromatin that is not used for the chromosomes. The structures which, in my opinion, are the true nucleoli have nothing to do with the formation of the chromosomes for the first polar spindle, as they are never connected with the chromosomes in any way and can be traced step by step until they are absorbed by the cytoplasm of the egg after the spindle is completely formed.

Many of Carnoy and Lebrun's illustrations of the formation of the first polar spindle in the egg of *Bufo vulgaris* are strikingly like my own, but we differ somewhat in our interpretation of them. According to their view, when the germinal vesicle in the egg of *Bufo vulgaris* migrates towards the upper pole, and before the nuclear membrane disappears, the paired chromatin filaments (which are exactly like those I find in *Bufo lentiginosus* during the same period) break up into small granules which cannot be distinguished from the granules of karyoplasm. *All the nucleoli suffer the same fate as the chromosomes excepting about ten which remain to form the chromosomes of the first polar spindle.* The karyoplasm meanwhile, forms a pronounced radiation from the "plage fusoriale" at the lower pole of the germinal vesicle. "Les nucléoles prédestinés montent le long des fila-

ments" and are carried to the "plage fusoriale" where they either become vacuolated in the center and form a ring, or else they fuse into one large mass and later regain their individuality. When the spindle is first formed, the chromosomes are very irregular in shape and there are distinct asters at the spindle poles which never contain a centrosome.

In the egg of *Bufo lentiginosus*, I have traced the chromosomes of the germinal vesicle up to the stage where the ends of a pair of chromosomes unite to form a closed ring. After this time, although I have had an abundance of material and have searched very carefully through every section of the germinal vesicle in a large number of eggs, I have been unable to find any trace of the chromatin. There is, I believe, no doubt but that the chromatin rings break up into minute granules which may, possibly, be carried by the karyoplasmic radiation to the lower pole of the germinal vesicle where they later form the chromosomes of the first polar spindle. I have never seen anything in this egg, however, that would indicate that some of the *nucleoli* are destined to form the chromosomes of the first polar spindle. A large number of nucleoli are always present throughout the early stages of maturation and they all appear to be undergoing the same processes of disintegration. Carnoy and Lebrun might consider the large irregular masses shown in Fig. 2 to be nucleoli in the general sense in which they seem to use the word, but these masses have been formed by the fusion of smaller chromatin granules (Fig. 1) and are in no way connected with the true nucleoli of the germinal vesicle.

Carnoy and Lebrun have followed the details of the formation of the first polar spindle and the later changes of the chromosomes much more carefully in the egg of *Triton* than in any of the other amphibian eggs they have studied. Their account of this form agrees substantially with that of *Bufo vulgaris* as regards the breaking down of the germinal vesicle, with the important exception that in *Triton*, *all* of the nucleoli are absorbed by the cytoplasm, none of them are reserved, as in *Bufo Vulgaris*, to form the chromosomes of the first polar spindle. The chromatin threads which were resolved from the nucleoli at an earlier period, break up into very small granules when the membrane

of the germinal vesicle disappears. The twelve chromosomes which later arise *from a coalescence of the chromatin granules* are at first very irregular in shape and they are scattered all along the spindle fibers; subsequently they undergo a double longitudinal division in preparation for the giving off of the polar bodies. Any chromatin not used for the chromosomes is absorbed by the cytoplasm.

Although there are always twelve chromosomes on the first polar spindle in the egg of *Triton*, Carnoy and Lebrun find only 8–10 chromosomes in the equatorial plate of the first polar spindle in the egg of *Bufo vulgaris*, and but 4–5 chromosomes at each pole just previous to the giving off of the first polar body. The failure of these investigators to find the definite number of chromosomes that must be present unless the egg of *Bufo vulgaris* is a marked exception to the rule that the number of chromosomes is constant for a given species, may possibly be accounted for on the supposition that some of the chromosomes were lost when the eggs were sectioned or that the sections of the egg were made so thick that some of the chromosomes were not visible.

In a more recent paper, Lebrun (13) gives the results of a re-examination of the maturation processes in the egg of *Triton*. He states that the double longitudinal division of the chromosomes does not take place in the complicated manner previously described by Carnoy and Lebrun, but according to the scheme represented by my text-figures 1–5. The late maturation changes in the egg of *Triton* are, therefore, strikingly similar to those I have found taking place in the egg of *Bufo lentiginosus*. Lebrun still believes that in the eggs of *Rana temporaria* and of *Bufo vulgaris* a certain number of the nucleoli are reserved to form the chromosomes of the first maturation spindle. A reëxamination of the maturation stages in the eggs of these amphibians would probably show that in these forms also the chromosomes are derived from fused masses of chromatin granules and that they have no connection whatever with the true nucleoli.

I have examined a large number of the eggs of *Bufo* at the stages of Figs. 3–4 and I can see no reason for believing with Carnoy and Lebrun that a division of the chromosomes

place at this time. During this period the chromosomes are exceedingly varied in size and shape. If the chromosome is oblong, it may have either its long or its short axis parallel with the longitudinal axis of the spindle; if the chromosome is pyramidal in shape, either the base or the apex of the pyramid may rest on the spindle fibers. I regard all of the changes in the shape of the chromosomes up to the stage of Fig. 7 as due solely to a rearrangement of the chromatin material preparatory to the later divisions. The first indication of any division of the chromosomes is the longitudinal splitting of the lateral wings which in some few cases can be found before the disappearance of the polar arms (Fig. 7). The apparent separation of the lateral wings at *X*, Fig. 11, I consider to be due to the fact that the angle of the V-shaped chromosome was cut off in sectioning. It very frequently happens that portions of one or of several chromosomes on a spindle are removed in this way. Sometimes, as in Fig. 4, the median knob of a chromosome is lacking; sometimes, the lateral wings have been removed (Figs. 6, 7). In rare instances the cut off portion of the chromosome will be found in the next section of the egg; but as the chromosomes are quite small a careful examination of the following sections often fails to disclose the missing part.

As found to be the case in many eggs besides that of *Bufo*, for example in *Cerebratulus* (Coe), *Polychœrus caudatus* (Gardiner), *Thalassema* and *Zirphœa* (Griffin), and *Triton* (Carnoy and Lebrun), all the chromatin of the germinal vesicle does not go to form the chromosomes of the first polar spindle, some of it is thrown out into the cytoplasm where it degenerates and sooner or later completely disappears. Even in the segmentation stages of the egg of *Ascaris*, Boveri (1) found that some of the chromatin is thrown out of the nucleus and absorbed by the cytoplasm. In all these cases there is obviously a mass reduction of the chromatin in preparation for the succeeding division of the cell. It may be, as suggested by Gardiner, that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter cells, and the other food for the cytoplasm." This theory would explain the facts as we now know them, but it cannot be proved until some stain can be found to differentiate the two sorts from each other.

Carnoy and Lebrun find a double division of the chromosomes in the egg of *Triton*, and they state that there is no reason why a longitudinal division of the chromosomes should not be a reduction division in the Weismann sense, in that it may separate the chromosome into two parts each containing different kinds of granules: it is certainly true if we admit a difference in the properties of the elementary granules. As all of the chromatin granules do not go into the chromosomes of the first polar spindle, there is a process of selection in the formation of the chromosomes and their subsequent division would be a permanent source of variation for the descendents.

The chromosomes of the first polar spindle in the egg of *Bufo lentiginosus* are at first exceedingly varied in shape; they may be round, triangular, or oblong. At this time it is obvious that they have no definite longitudinal axis. At the stage of Fig. 5 the chromosomes have elongated and lie parallel with the longitudinal axis of the spindle. When the wings have formed, there is a stage when the arms of the chromosomes are all approximately of the same length (Fig. 6). Is there a definite longitudinal axis at this time? If the part of the chromosome resting upon the spindle fibers is considered to be the longitudinal axis, then later this same axis is not only shorter than the transverse axis, but it practically disappears at the stage of Fig. 9. If shown Fig. 9 without the preceding figures, no one, I am sure would call the thickness of the chromosome at the angle of the V the longitudinal axis of the chromosome, and the division indicated in Fig. 11 would unhesitatingly be called a longitudinal division. If one arbitrarily states that the polar arms of the chromosomes in Fig. 5 form the true longitudinal axis, not only in this particular stage, but until division is completed, then the splitting seen in Fig. 11 is a transverse division, as is also the second division which takes place in the same direction. On the other hand, if the longer axis of the chromosome at the time when division occurs is considered to be the true longitudinal axis, then there is a double longitudinal division of the chromosomes and the egg of *Bufo* is thus brought into line with other amphibian eggs that have been studied. It would seem, as suggested by Sebaschnikoff (14), that the distinction between transverse

and longitudinal divisions of the chromosomes is not as important as many investigators have claimed: the division of the chromatin substance would appear to be the important thing, the manner of its achievement quite secondary, as Hertwig (9) has maintained.

There is, however, the following possibility to be considered. When the germinal vesicle breaks down, all of the chromosomes are arranged in pairs, in some cases the ends of a pair of chromosomes have united to form a closed ring. Very soon after this stage the chromosomes break up into granules and all traces of the chromatin substance is lost until innumerable chromatin granules appear in connection with the first polar spindle. It is conceivable that all of the chromatin granules belonging to a pair of chromosomes have remained united during this period of the apparent disintegration of the chromosomes, although I have not been able as yet to demonstrate such a union. If such is the case, then the chromosomes of the first polar spindle are bivalent structures, each being composed of the two chromosomes that had become paired at an earlier period of development. On this assumption it is probable that the knob-like thickening in the middle of the chromosomes, shown in Figs. 4 and 5, is caused by the fusion of the ends of the two chromosomes. In text-figure 1, *ABC* and *ACD* would represent the two chromosomes united at *AC*. The subsequent changes in the shape of the chromosomes serve merely to again elongate the original chromosomes (Text-fig. 3) which are finally separated by the division through *AC*. The first maturation division, therefore, is a reduction division and the second division only is a longitudinal one. It certainly cannot be mere chance that at the time of the breaking down of the germinal vesicle, the chromosomes should invariably become arranged in pairs. In light of the most recent investigations on spermatogenesis and ovogenesis it would seem as if the above explanation must be the true one for the maturation divisions in the egg of *Bufo*, although at present I am not able to prove it. I hope that the work I am doing on the spermatogenesis of this amphibian will throw some light on the maturation divisions in the egg.

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'99 The Maturation, Fertilization, and Early Development of the Planarians. Trans. of the Conn. Acad., Vol. X., 1899.

Explanation of Plate.

All figures were drawn with the aid of a camera lucida under a Zeiss Apoc. 2 mm., Oc. 4.

1. An early stage in the formation of the first polar spindle before the chromatin granules have fused into large masses.

2. A stage about one-half hour later than Fig. 1. The spindle has become barrel-shaped and the chromatin granules are fusing into large masses to form the chromosomes.

3. Twelve irregularly shaped chromosomes have differentiated from the chromatin masses and lie scattered along the spindle.

4. Spindle parallel to the surface of the egg. The chromosomes have elongated and many of them show a median knob. *C.M.*, chromatin mass outside the spindle.

5. About the same stage as Fig. 4. Chromosomes of very different sizes are found on the spindle.

6. Typical "oiselet" stage.

7. Chromosomes in various stages of development on the same spindle. In some of the chromosomes the splitting for the first maturation division can be seen while the polar arms are still present.

8. Section showing the growth of the lateral arms of the chromosomes at the expense of the polar arms.

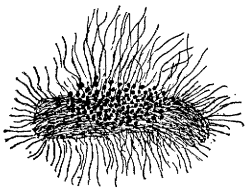
9. The V-shaped chromosomes after the disappearance of the polar arms.

10. An equatorial section of a spindle at the stage of Fig. 9. All twelve chromosomes are present.

11. Equatorial section. The notched ends of some of the chromosomes indicate the direction of the first maturation division.

12. Equatorial section. The first maturation division is completed and the second maturation division is indicated in some cases.

1



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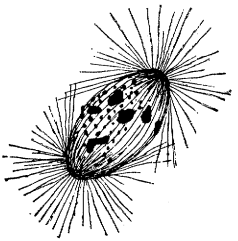

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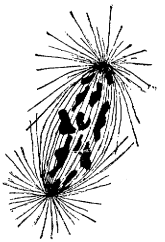

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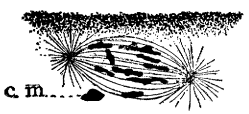

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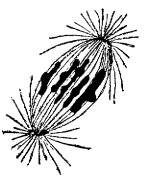

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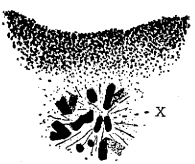

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